

## LETTERS TO THE EDITOR

### *The Pressure Inside Red Cells and the "Metabolic Pump"*

Dear Sir:

In a previous communication to this *Journal* (1) we reported results on the mechanical properties of the human red cell membrane which showed that the "stiffness" of the membrane decreased when the normally shaped cell was crenated in 1.2 per cent NaCl hypertonic solution. This decrease in stiffness, along with a change of intercept on a plot of critical pressure *versus* the reciprocal of the pipette radius (Figs. 9 and 10 in reference 1), was interpreted as meaning that there was a 2 mm water pressure difference, higher inside, across the membrane of the normally shaped cell. This pressure was reduced to zero when water was withdrawn from the cell as it was placed in the hypertonic medium. It is interesting to consider what role this pressure difference could play in terms of transport phenomena across cell membranes. Teorell (2) for example, has shown that many complex dynamic phenomena occurring across artificial membranes are explained only when a pressure difference across the membrane is included as one of the driving forces. The phenomena are very reminiscent of those of excitable membranes. Baker *et al.* (3) have shown that in perfused nerve axons, only within a certain range of perfusing pressures will normal axon shape and function be maintained. First, a pressure difference could be a force driving molecular species through a cell membrane. Second, since the functioning membrane is a closed surface, a pressure may affect the structural components of the membrane, for example, by maintaining them in a state of tension, thereby possibly affecting permeabilities of the membrane. The experiments reported here were undertaken to investigate any possibility of a relationship between the metabolic transport of the red cell and this pressure difference.

Tosteson and Hoffman (4) have studied the differences in regulation of cell volume between sheep cells of different genetic strains, those which have a high potassium content (*HK*) and those with a relatively lower potassium content (*LK*). In the *HK* cells, a cationic pump is working four times as fast and ATPase activity is at a higher level compared to the *LK* cells. These two types of cell, therefore, provide good material for testing any relation between active transport and pressure. A comparison is made of the deformability and pressure of *HK* and *LK* cells and of *HK* cells with the "pump" working and with it inactivated with ouabain.

The technique for measuring the deformability of the red cell has been described (1). A few drops of whole sheep blood from a jugular puncture were bled into standard medium or into standard medium with  $5 \times 10^{-6}$  M ouabain. The hematocrit was kept very low, approximately (0.05 per cent) so that single cells could be studied, and at this dilution no anticoagulant was required. The standard medium contained 140 mM Na, 5 mM K, 125 mM Cl, 9.4 mM  $\text{HPO}_4$ , 1.6 mM  $\text{H}_2\text{PO}_4$ , 200 mg per cent glucose and this was buffered at pH 7.32. Cells from *HK* and *LK* cells were drawn in the morning and used for periods of up to 10 hours.

Figs. 1 and 2 show plots of the critical pressure *versus* the reciprocal of the radius

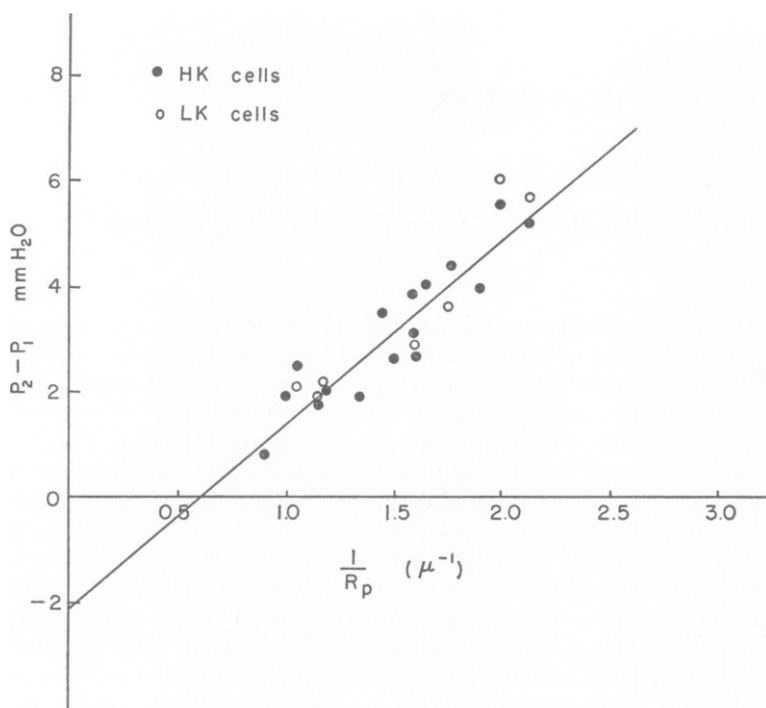


FIGURE 1 Plot of "critical pressure" required to produce instability of the "meniscus" of a portion of a red cell down into micropipettes of different radius. The line is the calculated regression line, including all the points. The intercept is significantly different from zero. No significant differences are seen between *HK* and *LK* cells.

of the pipette for *HK* and *LK* cells and for *HK* cells with the cationic pump working, and with it inactivated with  $5 \times 10^{-8}$  M ouabain, respectively. The statistical evaluation of these plots, which are similar to those in Figs. 9 and 10 in reference (1), is shown in Table I and is based on linear regression. This evaluation shows first that the intercepts

TABLE I  
STATISTICAL EVALUATION OF THE PARAMETERS (INTERCEPT AND SLOPE) OF FIGS. 1 AND 2 ASSUMING LINEAR REGRESSION

	<i>HK</i> cells	<i>LK</i> cells	<i>HK</i> in standard medium	<i>HK</i> ouabain
Intercept				
Intracellular pressure (mm H <sub>2</sub> O $\pm$ SE)	$-1.94 \pm 0.50$	$-2.40 \pm 0.54$	$-1.79 \pm 0.48$	$-2.28 \pm 0.63$
Slope				
$2 \times$ stiffness (dynes/cm $\pm$ SE)	$0.034 \pm 0.004$	$0.037 \pm 0.006$	$0.032 \pm 0.006$	$0.034 \pm 0.008$

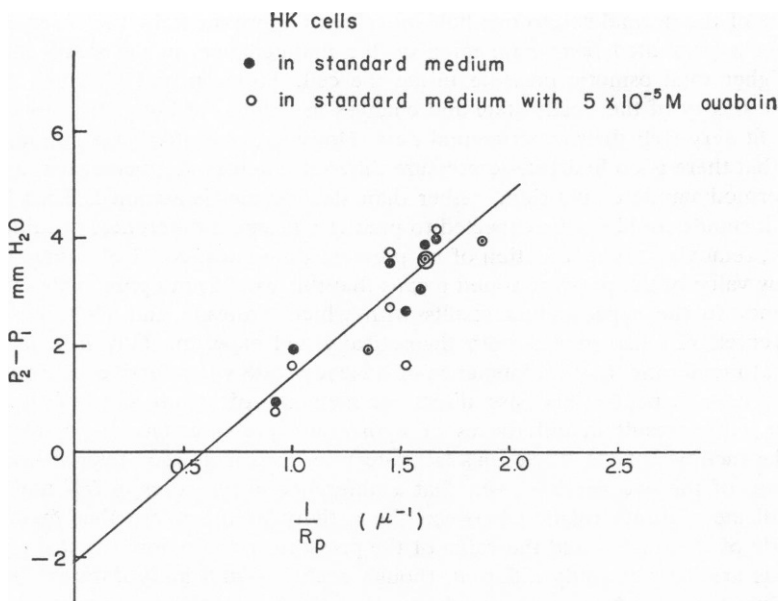


FIGURE 2 As in Fig. 1, but for *HK* cells only, with and without inactivation of the pump by ouabain. No significant differences were found either in slope or intercepts or the regression lines.

on the pressure axis of all four lines are insignificantly different from each other but are all significantly different from zero. An intercept which is different from zero and negative has been interpreted as indicating that there is a positive hydrostatic pressure inside the cell (1). The evaluation shows secondly that the slopes of all four lines are insignificantly different from each other. The slope of the line is a measure of the stiffness of the membrane, equivalent to the surface tension of a fluid interface but including rigidity of the membrane as well (1). In the case of human cells it has been shown that decreases in both pressure and stiffness could be detected when water was withdrawn from the cells when they were placed in hypertonic medium. These present results show that the mechanical properties of *HK* and *LK* sheep cells are similar to each other and that there is probably a hydrostatic pressure difference of approximately 2 mm water, higher inside, across the cell membrane of *both* these types of cell. Further, for *HK* cells, the mechanical properties of the membrane and the pressure difference across it do not change, within 10 hours, when the cation "pump" is inactivated with ouabain.

Since the pressure difference across the cell membrane is not immediately dependent on the cation pump it is probable that this slight pressure reflects a difference in osmotic pressure between the inside and outside of the cell. 2 mm water pressure is equivalent to the osmotic pressure of a concentration difference of  $10^{-5}$  moles. Solomon and Sidel (5) have measured the water permeability of the red cell membrane both for a diffusion gradient and for an osmotic gradient, the values being  $0.62 \times 10^{-14}$  and  $1.5 \times 10^{-14}$  ml  $H_2O$  per second per cm water pressure per red cell, respectively. On this basis, a 2 mm higher water pressure inside the cell would drive water out at a rate which would reduce

the volume of the normal cell to one-half its value in approximately 2 or 3 seconds. This presumably is prevented from happening by the maintenance, in the steady-state, of a slightly higher total osmotic pressure inside the cell. Tosteson and Hoffman (4) have produced a theory of the steady-state and changes in volume of both *HK* and *LK* sheep cells, that fit very well their experimental data. However, one of the assumptions of the theory is that there is no hydrostatic pressure difference across the membrane, and in addition, thermodynamic equilibrium, rather than steady-state, is assumed. Therefore, this theory as it stands could not be expected to predict a pressure difference. Modification of the theory, removing the assumption of no pressure difference, could of course be made, but the low value of the pressure found means that this would not appreciably change the fit of theory to the experimental results with which Tosteson and Hoffman are concerned. Teorell (2) has shown, both theoretically and experimentally in a model with an artificial membrane, that maintenance of a steady-state where active transport occurs across the membrane (in his case direct maintenance of steady current through the membrane) does result in differences of hydrostatic pressure. Our hope, then, in accepting the facilities of Dr. Tosteson's laboratory to investigate the pressure inside sheep erythrocytes of the two varieties, was that a difference in pressures in *HK* and *LK* cells would indicate a direct relation between the activity of the pump that maintains the steady-state of these cells, and the value of the pressure. Our finding is that the pressures in the cells are insignificantly different, though each is significantly different from zero. This finding does not, however, exclude the hypothesis that the active transport system (the pump) underlies the existence of the pressure. The logical possibilities would seem to be:

(a) The pump has little to do with the existence of the pressure, which may depend, for example, upon an attractive force between opposite faces of the erythrocyte, tending to reduce the volume of the cell and resisted by an elastic tension in the membrane and a positive hydrostatic pressure. Alternatively,

(b) The activity of the pump is basically the reason for the pressure, but though its activity is much less in the *LK* cells than in the *HK* cells, there is a compensatory difference in the leak rates of the two types of cell that results in the same steady-state pressure, reflecting, presumably, the maintenance of slightly higher osmotic pressure inside both types of cell.

The demonstration of the connection between the pumping and the pressure by inhibiting the pumping rate with ouabain is inconclusive, since the data (Tosteson and Hoffman, 4) on the volume changes resulting from this inhibition indicate that it takes as many as 60 hours before a new steady-state is reached.

We conclude that it is of great interest that both types of sheep cells have approximately the same pressure within the cell, and of the same magnitude as in the human cell, but the results do not answer the original question as to the basic reason for this pressure.

We take great pleasure in thanking Dr. D. C. Tosteson and members of his department at Duke University for so hospitably providing space and facilities to carry out these experiments. This research was supported under a Grant from the Life Insurance Medical Research Fund.

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## *Non-Planar Distribution of Nitrogen Atom Bonds in Nucleosides*

Dear Sir:

In a recent paper from this laboratory (1) it was pointed out that in some nucleosides the atoms of the base and the atoms substituted on the base were not coplanar. In particular, in the case of 5-fluoro-2'-deoxy- $\beta$ -uridine (FUDR) atoms N1 and C1' were out of the plane of the other atoms in the pyrimidine ring.<sup>1</sup> In calcium thymidylate C1' and O2 were both out of the plane of the base and on opposite sides of that plane (2). In adenosine-5'-phosphate, C1' was out of the plane of the base (3). In the paper on FUDR it was suggested that most of these displacements were due to steric hindrance between neighboring atoms. However, it was not possible to explain the displacement of N1 from the plane of the base in FUDR in this way.

Results recently obtained in this laboratory have prompted a revision of our view of the importance of steric hindrance in explaining these displacements. These results are as follows:

In 5-iodo-2'-deoxy- $\beta$ -uridine it was found that C1' was in the plane of the ring but O2' was out of the plane by 0.07 Å (4 standard deviations) (4). In addition, N1 and N3 were out of the mean plane, each by 0.04 Å (3 standard deviations) and on the same side of the plane.

In the rubidium salt of 5-fluoroorotic acid N1 and N3 were accurately in the mean plane of the pyrimidine ring but C2 was out of the plane by 0.09 Å (3 standard deviations) and O2 was out of the plane by 0.18 Å (6 standard deviations) (5).

All of these observations could be explained if the nitrogen atom were to form bonds which were not in the same plane; *i.e.*, if the spatial distribution of bonds formed by the nitrogen atom were intermediate between that expected for an atom in  $sp^3$  hybridization and that expected for an atom in  $sp^2$  hybridization.

It is not yet clear under what conditions the nitrogen atom will adopt this pyramidal distribution of bonds. It should be emphasized that nitrogen atoms do not invariably assume this configuration, since in thymine monohydrate (6) and in azaguanine monohydrate (7) the atoms of the base are strictly coplanar.

<sup>1</sup> The atom numbering system used in this communication is the same as that used in reference 1.